Date: 05/01/14 People in lab: Levi Palmer

Title: Preparation of PAO1 P. aeru frozen stocks

Start Time: 2:00 PM

Purpose: To preserve P. aeru bacterial strain PAO1 for future use

Protocol: LTM ed. 2 pg 68

Products:

Sample Label	Description	Source Label	Quantity
P. aeru PAO1 5/1/14 stock	PAO1 in glycerol	PAO1 BC P aeru	3

Notes: In -80C freezer

Stop Time: 2:30 PM

Next: Genomic Prep of P aeru DNA.

Date: 5/28/14 People in lab: Emily Puleo, Caleb Treccazi

Title: PCR of hmp to add prefix and suffix

Start Time: 10:35 am

Purpose: Amplify hmp with iGEM prefix and suffix

Protocol: LTM ed. 2 PCR

Exceptions: 7.5 uL template E Coli DNA, 2.5 uL primers

Products:

Sample Label	Description	Source Label	Quantity
PCR hmp 5/28 A	hmp with prefix and suffix at 55 C annealing temp	---	1
PCR hmp 5/28 B	hmp with prefix and suffix at 55 C annealing tmep	---	1

Results:

Notes: Accidentally put products in freezer during GE before TOPO cloning – CTZ

Stop Time: 2:30 pm

Next: Gel Electrophoresis to check products

Date: 5/28/14 People in lab: Emily Puleo, Caleb Treccazi

Title: Gel Electrophoresis of PCR hmp 5/28 A and B

Start Time: 2:30 pm

Purpose: Check for product

Protocol: LTM Ed. 2 Gels

Exceptions:

Products:

Well	1	2	3	4	5	6	7	8
-	-	Ladder	-	PCR hmp 5/28 A	-	PCR hmp 5/28 B	-	

Products:

Results:

Notes: Used 90 mA for ~35 min

Stop Time: 3:40 pm

Next: TOPO

- Ladder - PCR hmp 5/28 A - PCR hmp 5/28 B - -

Date: 5/28/14 People in lab: Emily Puleo, Caleb Treccazi

Title: TOPO Cloning PCR hmp products from 5/28 and transform

Start Time: 3:40 pm

Purpose: Make more products

Protocol: Invitrogent TOPO cloning kit manual and LTM Ed. 2 Chemical Transformations

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
hmp TOPO 5/28 A1-1	Plated transformed cells on xgal amp plate 20 uL	PCR hmp 5/28 A	1
hmp TOPO 5/28 B1-1	Plated transformed cells on xgal amp plate 20 uL	PCR hmp 5/28 B	1
hmp TOPO 5/28 A1-2	Plated transformed cells on xgal amp plate 200 uL	PCR hmp 5/28 A	1
hmp TOPO 5/28 B1-2	Plated transformed cells on xgal amp plate 200 uL	PCR hmp 5/28 B	1
hmp TOPO 5/28 A2-1	Plated transformed cells on xgal amp plate 20 uL	PCR hmp 5/28 A	1
hmp TOPO 5/28 B2-1	Plated transformed cells on xgal amp plate 20 uL	PCR hmp 5/28 B	1
hmp TOPO 5/28 A2-2	Plated transformed cells on xgal amp plate 200 uL	PCR hmp 5/28 A	1
hmp TOPO 5/28 B2-2	Plated transformed cells on xgal amp plate 200 uL	PCR hmp 5/28 B	1

Results:

Notes: Sample PCR hmp 5/28 A1 was melted in the hot water bath and the sample was vaporized; 15 uL 1000x xgal on amp plates for second batch; different ages of plates used for each batch

Stop Time: 7:15 pm

Next: Check growth and innoculate in LB+amp

Date: 5/29/14 People in lab: Emily Puleo, Caleb Treccazi

Title: Checking plates from 5/28

Start Time: 11 am

Purpose: Screen plates for correct colonies from 5/28

Protocol:

Exceptions:

Products:

Results: No growth

Notes: Possible problemw with resistance or something wrong with xgal or a problem with freezing PCR product before TOPO

Stop Time: 11:15 am

Next: Try PCR again without accidental freezing and plate on both xgal amp and just amp plates to test resistances

Date: 5/29/14 People in lab: Emily Puleo, Caleb Treccazi

Title: PCR of hmp to add prefix and suffix

Start Time: 11:20 am

Purpose: Amplify hmp with the iGEM prefix and suffix

Protocol: LTM Ed. 2 PCR

Exceptions: 7.5 uL template, 2.5 uL primers

Products:

Sample Label	Description	Source Label	Quantity
PCR hmp 5/29 A	hmp with iGEM prefix and suffix	---	1
PCR hmp 5/29 B	hmp with prefix and suffix	---	1

Results:

Notes:

Stop Time: 2 pm

Next: Gel electrophoresis to check product

Date: 5/29/14 People in lab:Caleb Treccazi

Title: Gel Electrophoresis of PCR hmp 5/29 A and B

Start Time: 2:05 pm

Purpose: To check for successful products

Protocol: LTM Ed. 2 Gels

Exceptions:

Products:

Well	1	2	3	4	5	6	7	8
-	-	Ladder	-	PCR hmp 5/29 A	-	PCR hmp 5/29 B	-	

Products:

Results:

Notes: Used 90 mA for ~45 min

Stop Time: 2:55 pm

Next: TOPO

Date: 5/29/14 People in lab: Caleb Treccazi

Title: TOPO cloning of PCR hmp 5/29 A and B

Start Time: 3:05 pm

Purpose: To make copies of successful products

Protocol: Invitrogen TOPO kit instruction manual

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
TOPO A 5/29	hmp product cloned	PCR hmp 5/29 A	1
TOPO B 5/29	hmp product cloned	PCR hmp 5/29 B	1

Results:

Notes: Cloned for ~ 9 min

Stop Time: 3:15 pm

Next: Chemical Transformations

Date: 5/29/14 People in lab: Caleb Treccazi

Title: Chemical Transformation of TOPO A and B 5/29

Start Time: 3:20 pm

Purpose: To grow and screen cells with hmp with the prefix and suffix

Protocol: LTM Ed. 2

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
CT 1 5/29	20 uL CT cells on amp xgal plate	TOPO A 5/29	1
CT 2 5/29	200 uL CT cells on amp xgal plate	TOPO A 5/29	1
CT 3 5/29	20 uL CT cells on amp xgal plate	TOPO B 5/29	1
CT 4 5/29	200 uL CT cells on amp xgal plate	TOPO B 5/29	1
CT 5 5/29	20 uL CT cells on amp plate	TOPO B 5/29	1
CT 6 5/29	200 uL CT cells on amp plate	TOPO B 5/29	1

Results: CT 5 was discarded as contaminated after lawn growth, plates with colonies were saved from CT 1 - CT 4 for screening

Notes: Amp plates were unmarked or badly marked in the fridge but were assumed to be amp and usable.

Stop Time: 6:10 pm

Next: Save plates for isolations